SCALABLE EXPANSION OF HUMAN UMBILICAL CORD MATRIX- AND ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM/STROMAL CELLS AND DERIVED EXOSOMES IN THE SINGLE-USE, VERTICAL-WHEEL BIOREACTOR SYSTEM USING A HUMAN PLATELET LYSATE CULTURE SUPPLEMENT

Ana Fernandes-Platzgummer, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, and The Discoveries Center for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
ana.fernandes@tecnico.ulisboa.pt
Diogo de Sousa Pinto, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
Miguel de Almeida Fuzeta, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
Carlos A.V. Rodrigues, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, and The Discoveries Center for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
Nuno Bernardes, iBB-Institute for Bioengineering and Biosciences, Biological Sciences Research Group, Lisboa, Portugal
Sung-Hoon Jung, PBS Biotech Inc., Camarillo, CA, USA
Rong-Jeng Tseng, AventaCell Biomedical Corp., Ltd, New Taipei City, Taiwan
William Milligan, AventaCell Biomedical Corp., Ltd, Atlanta, Georgia, USA
Brian Lee, PBS Biotech Inc., Camarillo, CA, USA
Cláudia Lobato da Silva, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, and The Discoveries Center for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
Joaquim M. S. Cabral, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, and The Discoveries Center for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

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Mesenchymal stem/stromal cells (MSC) hold great promise for tissue engineering and regenerative medicine settings due to their multilineage differentiation potential and their intrinsic immunomodulatory and trophic activities. Large cell doses (>1x10^6 cells/kg) are however required for clinical implementation and the success in obtaining those cell numbers is dependent on efficient *ex vivo* expansion protocols able to comply with Good Manufacturing microcarrier-based cultures in scalable bioreactors using serum-/xenogeneic-free (S/XF) culture components. In this context, a S/XF microcarrier-based culture system was successfully established for the expansion of human UCM and AT MSC using an innovative disposable bioreactor system utilizing the Vertical-Wheel™ technology (PBS-0.1 MAG with maximum working volume of 100 mL, PBS Biotech) combined with a commercially available fibrinogen-depleted human platelet lysate-based culture supplement (UltraGRO™-PURE, AventaCell BioMedical). By optimizing the agitation and feeding regimes, UCM and AT MSC were successfully expanded to maximum cell densities of 5.3 ± 0.4 x 10^5 cell/mL (*n*=3) and 3.6 ± 0.7 x 10^5 cell/mL (*n*=3), respectively, after 7 days of culture (cell viability ≥ 94%), while maintaining their identity.

Recently, increasing evidence has proposed extracellular vesicles (EVs), as exosomes, as mediators of many of the MSC-associated therapeutic features. Exosomes are small EVs (30-150nm) of endocytic origin, involved in intercellular communication, through transfer of a cargo of proteins and RNAs. In this context, the platform established for the expansion of MSC is under optimization for exosome production. Dynamic culture systems, as the one presented herein, are expected to allow a higher exosome titer, as well as a better control when fine-tuning the exosomes' properties, by changing culture conditions (*e.g.* shear, oxygen). Preliminary results have shown that human MSC expanded in the Vertical-Wheel™ bioreactor system allowed to obtain a population of EVs with a more homogeneous size distribution profile, when compared to cells cultured in traditional static systems. Overall, we demonstrate that this culture system is able to robustly manufacture human MSC and MSC-based exosomes towards the development of novel therapeutic products.